*Biochemical Pharmacology*, Vol. 46, No. 2, pp. 307–310. 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00


# MODULATORY EFFECT OF HYPERTHERMIA ON HEPATIC MICROSOMAL CYTOCHROME P450 IN MICE

M. REZA ANARI and KENNETH W. RENTON*
Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

(*Received* 18 *January* 1993; *accepted* 8 *March* 1993)

**Abstract**—It is widely known that the clearance of drugs is often compromised during episodes of infectious disease via a down-regulation of cytochrome P450 (P450) at a pre-translational step in enzyme synthesis. Etiocholanolone (ETC), a potent inflammatory agent, induces fever in humans and causes a decrease in the clearance of certain drugs that are metabolized by P450. On this basis it is widely believed that the fever *per se* rather than the immune modulation that occurs during infections may have a major role in depression of microsomal P450 enzymes during viral infections in humans. In the present study, we demonstrated that although ETC did not induce hyperthermia in mice, it still evoked a depression of the levels of P450 in hepatic microsomes. Ethoxyresorufin *O*-deethylase (EROD) was also inhibited significantly when hepatic microsomes were incubated with various concentrations of ETC *in vitro*. P450 levels and EROD activities remained unchanged following hyperthermia that was induced by a non-inflammatory procedure using 2,4-dinitrophenol. Provided the response in rodents is similar to humans, these results indicate that the depression of drug biotransformation by ETC in humans is more likely to be caused by the direct effects of this agent or other mechanisms rather than by the fever it produces. This may suggest that the loss of drug metabolism in humans during infections is due to the activation of host defence responses rather than to the febrile nature of the illness.

The down-regulation of cytochrome P450 (P450†) during infectious disease or following the stimulation of host-defence responses has been reported widely [1, 2]. A loss in the capacity of liver to metabolize drugs during infection occurs in both animals and humans [3–6] and has been demonstrated to occur in animal species via the production of interferon and other cytokines and a loss in P450 at a pre-translational level [2, 7, 8]. Although it is likely that this mechanism also occurs in humans, it is a widely held belief that the loss in drug clearance observed clinically is caused by the hyperthermia that commonly accompanies these illnesses [9]. This idea is based on experiments with etiocholanolone (ETC), an inflammatory pyrogenic steroid [10], that decreases the clearance of a number of agents in humans. During ETC-induced fever, clearance of drugs such as antipyrine [11], salicylamide [12], and quinine [13] is decreased. As all of the reports on decreased drug clearance during viral illness in humans also reported the presence of fever [4, 6, 14], it has been concluded that hyperthermia was a major determinant in drug metabolism loss. On the other hand, drug biotransformation in animals is down-regulated during host defence activation even in the absence of fever [2], which raises questions about the role of fever in humans as the loss of drug clearance following the administration of etiocholanolone could have resulted from immune activation by this steroid or by a direct action of the drug itself.

In this study in mice, we investigated whether ETC has a direct effect on drug biotransformation in the absence of fever and attempted to determine if drug biotransformation can be down-regulated during a non-immune-mediated hyperthermia.

## MATERIALS AND METHODS

*Chemicals.* ETC (CAS 53-42-9), 2,4-dinitrophenol (DNP; CAS 51-28-5) and all other reagents were obtained from the Sigma Chemical Co. (St. Louis, MO).

*Animal treatment.* Adult male Swiss Albino mice (CD-1), 20–25 g, were obtained from Charles River Canada Laboratories, Montreal, Quebec. Mice were allowed to acclimatize for at least 6 days on clay chip bedding, with a 12-hr light/dark cycle and a 22 ± 1° room temperature. Diet consisted of Purina Rat Chow and water *ad lib*. DNP (25 mg/kg body wt in 0.1 mL normal saline, pH 7, s.c.) and ETC (30 mg/kg in 0.1 mL polyethylene glycol 300, i.p.) were administered as single doses. Control groups were injected with an equivalent volume of the vehicles. Rectal temperature was recorded with a digital microprobe thermometer model BAT-12 and RET-3 rectal probe for mice (Harvard Apparatus Co., South Natick, MA), which was inserted rectally to a depth of 20 mm. To adapt animals to the technique and minimize stress-induced hyperthermia, rectal body temperature was measured twice a day for 3 days before the pyrogen injections.

*Enzyme determinations.* Hepatic microsomes were prepared as previously described [15] and resuspended in 20% (v/v) glycerol phosphate buffer (0.2 M, pH 7.4) and stored at −80°. Protein was

* Corresponding author. Tel. (902) 494-3430; FAX (902) 494-1388.

† Abbreviations: P450, cytochrome P450; DNP, 2,4-dinitrophenol; ETC, etiocholanolone, etiocholan-3α-OH-5β-androstanedione; EROD, ethoxyresorufin *O*-deethylase; and DMSO, dimethyl sulfoxide.

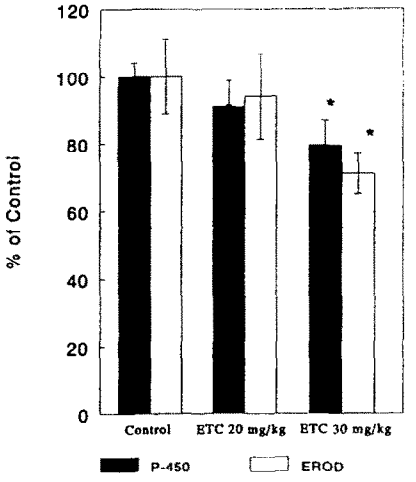


Fig. 1. Effect of ETC on hepatic total microsomal P450 and EROD activity *in vivo*. Mice (N = 4) were killed 24 hr after injection of ETC (20 and 30 mg/kg, i.p.). Microsomal enzymes were prepared and analyzed as described in Materials and Methods. The control value of P450 was 0.78 ± 0.13 nmol/mg protein and that for EROD 205 ± 23 pmol resorufin/mg protein/min. Values are means ± SEM. Key: (*) sigificantly different from control, $P < 0.05$.

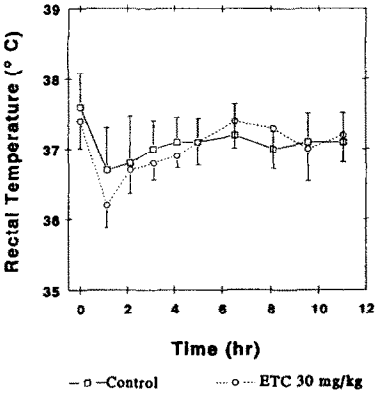


Fig. 2. Effect of ETC on body temperature. Rectal temperature was measured in all animals at time 0, after which four mice were injected with ETC (30 mg/kg, i.p.). The control group of four mice was injected with polyethylene glycol 300, i.p. Values are means ± SEM.

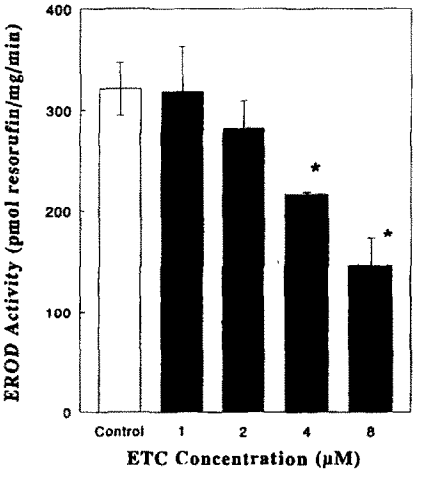


Fig. 3. Inhibitory effect of ETC on hepatic microsomal ethoxyresorufin O-deethylation *in vitro*. Pooled hepatic microsomes of two saline control mice were used with a final microsomal protein concentration of 0.125 mg/mL. ETC at various concentrations in DMSO was added to the microsomal suspension prior to the addition of substrate. Each value is the mean ± SEM of three replicates. Key: (*) significantly different from control, $P < 0.05$.

determined by the method of Lowry *et al.* [16], and P450 by the method of Omura and Sato [17]. Ethoxyresorufin *O*-deethylase (EROD) activity was determined by the method of Burke and Mayer [18] and likely represents a measure of several P450 isoenzymes in non-induced mice. ETC was dissolved in dimethyl sulfoxide (DMSO), which provided a final concentration of 61 mM per incubation.

*Statistical analysis.* One-way analysis of variance followed by the Scheffe's test were used for comparison amongst the multiple-treated groups and the relevant controls.

## RESULTS

*Effect of ETC on total P450, EROD activity and body temperature.* The effect of *in vivo* treatment with ETC on P450 and EROD activity in hepatic microsomes prepared from mice is illustrated in Fig. 1. P450 and EROD were decreased 24 hr following the administration of 30 mg/kg of ETC. No hyperthermia was observed over a 12-hr period following the maximum tolerable dose of ETC (30 mg/kg), as shown in Fig. 2. When hepatic microsomes were incubated *in vitro* with ETC, concentrations greater than 2 μM significantly inhibited EROD activity (Fig. 3). DMSO, which was used as a vehicle at the concentration of 61 mM in the *in vivo* studies, did not affect EROD activity.

*Effect of DNP on P450, EROD activity and body temperature.* The hyperthermic response of animals treated with DNP is illustrated in Fig. 4. DNP induced a rapid monophasic hyperthermic response reaching a mean elevation of 39° after 45 min that was maintained for at least 2 hr. DNP treatment did not produce any changes in the levels of P450 or EROD activity. The levels of P450 (0.8 ± 0.1 vs 0.7 ± 0.1 nmol/mg protein) and EROD activity (335 ± 16 vs 322 ± 12 pmol resorufin/mg protein/min) in microsomes prepared from animals treated with DNP for 24 hr were identical to those in microsomes prepared from control animals.

## DISCUSSION

In most reported cases of decreased drug biotransformation in humans during infectious

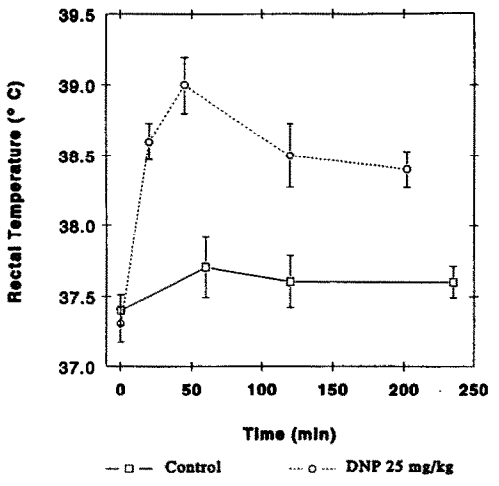


Fig. 4. Effect of DNP on body temperature in mice. Rectal temperature was measured in all animals at time 0, after which eight mice were injected with DNP (25 mg/kg, s.c.). The control group of eight mice was injected with saline, s.c. Values are means ± SEM.

disease, fever has been observed as an integral part of the illness [4, 6, 14, 19]. ETC, a potent inflammatory agent and pyrogen in humans, has been used widely in volunteers to evaluate the role of fever in drug disposition [11–13]. Elin *et al.* [11] demonstrated that in 14 out of 33 subjects, who met a defined minimum criteria indicating that fever had been evoked by ETC, the clearance of antipyrine (10 mg/kg) was impaired, but there was no overall correlation between the degree of fever and the loss in antipyrine clearance. When higher doses of antipyrine (18 mg/kg) were used in only three subjects, no fever occurred and no decrease in clearance of antipyrine was observed. Although this higher dose of antipyrine used in this study may have suppressed the fever, the elimination of quinine, which has no antipyretic effect, has also been shown to be impaired following ETC administration [13]. These observations in both natural infections and following ETC administration have led to the belief that fever plays a central role in P450 depression in humans during infectious disease. On the other hand, data from other species indicate that the loss in drug biotransformation occurs primarily as a result of host defence response activation [1, 2].

In the present study, our results indicate that ETC treatment does not induce a febrile response in mice but can lower the levels of P450 in the liver, and when ETC is incubated with microsomes *in vitro*, it can directly inhibit the metabolism of ethoxyresorufin by P450. This suggests that ETC has a direct effect on drug biotransformation capacity in the liver that is independent of an evoked hyperthermia. ETC and other pyrogenic steroids produce fever in the human but not in the rabbit, dog, cat, guinea pig, rat or mouse [20], which is consistent with the finding that ETC can release endogenous pyrogen from human leucocytes but not animal leucocytes *in vitro* [21]. The *in vivo* effect of ETC in humans may be mediated via interleukin-1 as ETC is a potent inducer of this inflammatory cytokine [22], which is a well known pyrogen [23]. Interleukin-1 can down-regulate P450 directly via a pre-transitional mechanism in animals [24]. In the original study by Elin *et al.* [11], only 14 subjects responded and the correlation between fever and drug metabolism loss was poor. It therefore seems likely that the down-regulation of P450 in humans following ETC administration is due to the effect of interleukin-1 and/or a direct effect of ETC on the enzyme and that the production of fever is concomitant but not responsible for the effect on drug metabolism.

We have also demonstrated that following a non-inflammatory-type fever induced by DNP, P450 and EROD activity were unchanged in hepatic microsomes. Similarly, Neville and Singh [25] reported that whole-body hyperthermia induced by a radiant heat device does not affect the total content of hepatic P450. The small but significant depression of aminopyrine N-demethylation reported by Neville and Singh [25] may be related to the induction of interleukin-1 [24] as whole-body hyperthermia is capable of inducing release of interleukin-1 *in vivo* [26].

Our results indicate that the pyrogenic steroid ETC that was used to invoke fever as a mechanism for the down-regulation of P450 during human infectious disease has direct effects on P450-mediated drug biotransformation. Androstanedione-derivatives like ETC have been shown to have a high binding affinity in their interactions with hepatic microsomal P450 compared with other steroids [27]. High binding affinity of ETC toward microsomal enzymes could lead to the occupation of available binding sites of P450 and inhibit binding of other substrates by a competitive mechanism or could deactivate P450 via a non-competitive mechanism. These observations strengthen the argument that ETC depresses P450 by a direct mechanism rather than via its pyrogenic properties. The lack of depression of P450 in non-inflammatory-type hyperthermia induced by DNP [28] also suggests that fever has little effect on drug biotransformation. We conclude that the loss in drug clearance that often accompanies infectious disease in humans is more likely due to the activation of host defence mechanisms than to the fever that accompanies the disease.

*Acknowledgements*—This work was supported by grants from the Medical Research Council of Canada. We are grateful to Dr Alastair E. Cribb for helpful discussions and technical assistance.

## REFERENCES

1. Mannering GJ and Deloria LB, The pharmacology and toxicology of interferons: An overview. *Annu Rev Pharmacol Toxicol* **26**: 455–515, 1986.
2. Renton KW and Knickle LC, Regulation of hepatic cytochrome P-450 during infectious disease. *Can J Physiol Pharmacol* **68**: 777–781, 1990.
3. Fleetham JA, Nakatsu K and Munt PW, Theophylline pharmacokinetics and respiratory infections. *Lancet* **2**: 898, 1978.
4. Forsyth JS, Moreland TA and Ryelance GW, The

effect of fever on antipyrine metabolism in children. *Br J Clin Pharmacol* **13**: 811–815, 1982.
5. Greenwald M and Koren G, Viral-induced changes of theophylline handling in children. *Am J Asthma Allergy Peds* **3**: 162–165, 1990.
6. Kraemer MJ, Furukarwa CT, Koup JR, Shapiro GG, Pierson WE and Bierman CW, Altered theophylline clearance during an influenza B outbreak. *Pediatrics* **69**: 476–480, 1982.
7. Craig PI, Mehta I, Murray M, McDonald D, Åström A, van der Meide PH and Farrell GC, Interferon down regulates the male-specific cytochrome P450IIIA2 in rat liver. *Mol Pharmacol* **38**: 313–318, 1990.
8. Morgan ET and Norman CA, Pretranslational suppression of cytochrome P450h (IIC11) gene expression. *Drug Metab Dispos* **18**: 649–653, 1990.
9. Oski FA, deAngelis CD, Feigin RD and Warshaw JB (Eds.), *Principles and Practice of Pediatrics*, pp. 203–204. J. B. Lippincot Co., Philadelphia, 1990.
10. Kappas A, Hellman L, Fukushima DK and Gallagher TE, The pyrogenic effect of etiocholanolone. *J Clin Endocrinol* **16**: 948–952, 1956.
11. Elin RJ, Vessel ES and Wolff SM, Effects of etiocholanolone-induced fever on plasma antipyrine half-lives and metabolic clearance. *Clin Pharmacol Ther* **17**: 447–457, 1975.
12. Song CS, Gelb NA and Wolff SM, The influence of pyrogen-induced fever on salicylamide metabolism in man. *J Clin Invest* **51**: 2959–2966, 1972.
13. Trenholme GM, Williams RL, Rieckmann KH, Frischer H and Carson PE, Quinine disposition during malaria and during induced fever. *Clin Pharmacol Ther* **19**: 459–467, 1976.
14. Clark CJ and Boyd G, Theophylline pharmacokinetics during respiratory viral infection. *Lancet* **1**: 492, 1979.
15. el Defrawy el Masry S and Mannering GJ, Sex-dependent differences in drug metabolism in the rat. II. Qualitative changes produced by castration and administration of steroid hormone and phenobarbital. *Drug Metab Dispos* **2**: 279–284, 1984.
16. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
17. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
18. Burke MD and Mayer RT, Ethoxyresorufin: Direct fluorometric assay of a microsomal *O*-dealkylase which is preferentially induced by 3-methylcholanthrene. *Drug Metab Dispos* **2**: 583–588, 1974.
19. Koren G and Greenwald M, Decreased theophylline clearance causing toxicity in children during viral epidemics. *J Asthma* **22**: 75–79, 1985.
20. Wolff SM, Kimball HR and Perry S, The biological properties of etiocholanolone. *Ann Intern Med* **67**: 1268–1295, 1967.
21. Bondy PK and Bodel P, Mechanisms of action of pyrogenic and antipyrogenic steroid *in vitro*. In: *Pyrogens and Fever* (Eds. Wolstenholme GE and Birch J), pp. 101–113. Churchill Livingstone, Edinburgh, 1971.
22. Watters JM, Bessey PQ, Dinarello CA, Wolff SM and Wilmore DW, The induction of interleukin-1 in humans and its metabolic effects. *Surgery* **98**: 298–306, 1985.
23. Dinarello CA, The endogenous pyrogen in host-defense interactions. *Hosp Prac* **15**: 111–128, 1989.
24. Wright K and Morgan ET, Regulation of cytochrome P450IIC12 expression by interleukin-1$\alpha$, interleukin-6, and dexamethasone. *Mol Pharmacol* **39**: 468–474, 1991.
25. Neville AJ and Singh G, Effect of whole body hyperthermia on hepatic cytochrome P-450. *Cancer Chemother Pharmacol* **25**: 342–344, 1990.
26. Neville AJ and Sauder DN, Whole body hyperthermia induces interleukin-1 *in vivo*. *Lymphokine Res* **7**: 201, 1988.
27. Estabrook RW, Martinez-Zedillo G, Young G, Peterson JA and McCarthy J, The interaction of steroids with liver microsomal cytochrome P-450, a general hypothesis. *J Steroid Biochem* **6**: 419–425, 1975.
28. Loomis WF and Lipmann F, Reversible inhibition of the coupling between phosphorylation and oxidation. *J Biol Chem* **173**: 807–808, 1948.